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Comparative Efficacy of Toltrazuril, Amprolium and Embazine Fort® against Mixed *Eimeria* Species Infection in Broilers

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SUMMARY

The efficacy of toltrazuril, amprolium, and Embazine Fort® (sulphaquinoxaline/diaveridine) against a mixed *Eimeria* species (*E. tenella*, *E. acervulina*) infection in broiler chickens housed in battery cages was evaluated. The chickens were divided into five groups of 20 birds each. Four groups of birds were each inoculated with 5×10^4 sporulated coccidia oocysts at day 14 of age, while one group served as uninfected control. At day 16 of age each bird in 3 infected groups was given toltrazuril, amprolium or Embazine Fort® as prescribed by manufacturers. Two groups (an infected and uninfected group) did not receive treatment. Treatment with toltrazuril, amprolium or Embazine Fort® prevented coccidiosis related mortality, improved weight gain and packed cell volume and reduced faecal scores when compared to infected/untreated control. The mean weight gain of the birds medicated with toltrazuril were significantly higher ($P < 0.05$) than either the amprolium or Embazine Fort® medicated birds. In conclusion, although amprolium and Embazine Fort® were efficacious, toltrazuril was superior and is thus recommended for therapy of coccidiosis in broilers.

Keywords: Toltrazuril, amprolium, Embazine Fort®, *Eimeria*, broilers.

INTRODUCTION

Coccidiosis is one of the most important diseases of poultry worldwide (Lee et al., 2009) and is caused by apicomplexan parasites of the genus *Eimeria* of which there are seven species affecting chickens. The disease is characterized by marked morbidity, mortalities, and reduction in productivity and feed conversion efficiency of affected chickens (Jang et al., 2007). It is a significant problem to the poultry industry through out the world and is responsible for 6-10% of broiler mortalities (Banfield et al., 1999). In spite of advances in immunological, biotechnological and genetic methods, control of coccidiosis chiefly depends upon prophylaxis

chemotherapy with anticoccidial drugs (McDougald and Reid, 1997). However, the emergence of drug resistance in coccidia is a major problem with most of the drugs, which in due course limit their use. Drug resistance in *Eimeria* species infections, has been reported against almost all compounds that have been introduced (Chapman, 1997). In Nigeria, commonly used coccidiostats include sulphaquinoxaline and pyrimidine derivatives such as amprolium (Oladoja and Olusanya, 2007; Anosa and Okoro, 2011).

Amprolium is a thiamine analogue used in treatment and prevention of coccidiosis in poultry and rabbits (McDougald and Reid, 1997). Sulphaquinoxaline belongs to the sulphonamide group and acts by competing for incorporation of PABA and metabolism of folic acid (McDougald and Reid, 1997). Amprolium and sulphaquinoxaline/diaveridine have been used extensively in Nigeria for over two decades and there have been claims of resistance to the two drugs in the field (Anosa and Okoro, 2011). Chapman (1997) has also reported widespread resistance to most drugs in use today including amprolium and sulphonamides. Toltrazuril is a symmetrical triazone and has no chemical relationship with other anticoccidial agents currently in the market. Although it has been used for several years in many countries it is not one of the commonly used drugs in Nigeria, hence the efficacy of toltrazuril against Nigerian field isolates have not been reported. Considering the proven efficacy of toltrazuril, it could serve as potential replacement for less effective anticoccidials in Nigeria.

In this study, the efficacy of toltrazuril, amprolium and Embazine Fort® against experimental mixed *Eimeria* species infection in broiler chickens was compared.

MATERIALS AND METHODS

Birds and management

One hundred Anak broiler chickens purchased at day old from a commercial hatchery were used for the study. They were kept in standard poultry houses in suspended wire meshed cages at a stocking density of approximately 13 birds per m³. The birds were fed *ad libitum* on a proprietary broiler ration (AMEn = 2,900kcal/kg, CP = 19%) and also given access to water *ad libitum*. At 2 weeks of age they were weighed and allocated to cages (20 birds per cage) by a restricted randomization procedure that approximately equalized initial weights. The birds were routinely vaccinated against Newcastle and Infectious bursal diseases. The birds were kept in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH publication no. 85-23; National Research Council, U.S.A (NRC) 1985).

Parasites

A suspension containing infective mixed *Eimeria* species oocysts was obtained from National Veterinary Research Institute (NVRI), Vom, Nigeria. To type (identify) the isolate, five 2 week old Anak broiler chicks reared coccidia free on wire floored cages were inoculated with 10⁴ (mixed *Eimeria* species) sporulated oocysts. At day 6 post-infection (PI), the birds were killed by cervical dislocation and examined for any post mortem lesions. The isolate was then differentiated on the basis of oocyst morphology, site of colonization, pathology and clinical signs according to the method of Long and Joyner (1976). The isolate contained *Eimeria tenella* (>90%) and *Eimeria acervulina* (<10%) oocysts. Sporulated oocysts in the oocyst suspension and inoculum were counted by the Haemocytometer method as described by Holdsworth *et al.* (2004).

Drugs and administration

Toltrazuril base solution (Toltralvet® 2.5%, Arab Pesticides and Vet. Drugs, Irbid, Jordan) was administered in drinking water at a concentration of 25 mg/l (0.0025% solution) for a period of 2 days as prescribed by manufacturers. Amprolium (Amprolium 250WSP®, Kepro B.V., Holland) was administered in drinking water at a concentration of 250 mg/l (0.025%) for a period

of 5 days as prescribed by the manufacturers. Embazine Fort® (active ingredients: sulphaquinoxaline and diaveridine) was obtained from Turn Right Ltd, Lagos, Nigeria and administered in drinking water (at a concentration of sulphaquinoxaline: 188 mg/l, diaveridine: 19.6 mg/l) for 5 days as prescribed by the manufacturers.

Experimental design

The birds were divided into 5 groups (A, B, C, D and E) of 20 chickens each at 14 days of age. Of these, birds of four groups (i.e. groups A-D) were inoculated with 5 x 10⁴ sporulated oocysts at day 14 of age. Group E birds were not inoculated. At 16 days of age (day 2 PI.) birds in group A were medicated with amprolium while birds of groups B and C received sulphaquinoxaline/diaveridine (Embazine Fort®) and toltrazuril respectively. Birds of groups D and E were not medicated.

Evaluation of efficacy

Mortality was recorded each day during the experiment. All birds that died in the course of the study were examined for intestinal lesions due to coccidiosis (McDougald and Reid, 1997).

All the birds from each of the groups were weighed at day 14 and at day 21 of age (i.e. day 7 PI). The bodyweight gains between days 14 and 21 of age were calculated and expressed in both grammes and percentages. A faecal score ranging from 1-4 was recorded from five birds selected randomly from each group of birds between days 18 and 22 of age (i.e. days 4-8 PI.). A score of 1 indicated normal faeces, and a score of 4 indicated the maximum departure of the faeces from normal. Scores of 1, 2, and 3 represented intermediate gradations (Morehouse and Baron, 1970).

Blood samples (0.2 ml) were collected by jugular venipuncture from each chicken in all groups at day 14 and again at day 21 of age. The packed cell volume was determined by the microhaematocrit method (Campbell and Coles, 1986).

Statistical analysis

The data (on weight gains, faecal scores and packed cell volumes) obtained from the study were summarized as means ± standard error of means. Statistical comparisons between the

treatment groups were made by one way analysis of variance (1 way ANOVA), and means separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

There were two mortalities observed from the group D birds (Table I). At necropsy they revealed gross lesions typical of coccidiosis (ballooning of the large intestines and petechial haemorrhages of the serosal surface of the large intestine, thickening of the wall of the caeca with a blood filled lumen). There were no mortalities from groups A, B and C birds. The ability of amprolium, Embazine Fort® and toltrazuril to prevent mortality in the medicated groups of bird shows that the drugs were efficacious in the control of coccidiosis. Some workers suggest that mortality is the primary criterion of measuring efficacy, and the ratios of mean weight gains and faecal scores are used as indicators of morbidity (Morehouse and Baron, 1970). The occurrence of deaths due to coccidiosis is the most reliable sign of resistance to anticoccidials, especially for the highly pathogenic species *E. tenella* and *E. necatrix* (Bedrnik, 1983). Mortality, despite treatment is therefore a very good indicator of resistance problems on the field (Stephan et al., 1997).

TABLE I: Effect of amprolium, Embazine Fort® and toltrazuril on mortality, mean weight gains and fecal scores in experimental mixed *Eimeria* species infection in broilers

Group	Mortality		Weight Gain		Mean fecal score
	x/y	%	g	%	
A	0/ 20	0	341.5±10.58 ^a	93.1	1.60±0.24 ^a
B	0/20	0	346.2±6.26 ^a	94	1.80±0.24 ^a
C	0/20	0	360.7±4.23 ^b	98	1.40±0.24 ^a
D	2/20	10	290.7±4.30 ^c	79	3.40±2.72 ^b
E	0/20	0	370.6±8.52 ^d	100	0.00±0.00 ^c

Different superscripts within a column indicate significant difference between the means, abcd: p<0.05.

TABLE II : Effect of amprolium, Embazine Fort® and toltrazuril on PCV in experimental mixed *Eimeria* species infection in chickens

Group	Day 14 old (Day of infection)	Day 21 old (Day 7 P.I.)
A	28.61±0.39 ^a	25.64(0.41) ^a
B	28.41±0.27 ^a	26.90(0.60) ^a
C	27.98±0.51 ^a	26.38(0.39) ^a
D	28.69±0.47 ^a	23.59(0.76) ^b
E	27.89 ±0.36 ^a	28.06(0.15) ^c

Different superscripts within a column indicate significant difference between the means, abcd: p<0.05. P.I. = Post infection.

The mean body weight gain of group D birds (290.7 g) was significantly lower than the group E birds (370.6 g). The mean weight gains of group A (341.5 g) and group B (346.2 g) birds were significantly lower than the group C (360.7 g) birds (Table I). The results therefore indicate that toltrazuril was more effective in improving mean weight gains when compared to the unmedicated and infected group D. In addition, toltrazuril was also more efficacious than amprolium and Embazine Fort® in improving mean weight gains. The results of this study agree with those of Ramadan et al. (1997) who showed that the addition of toltrazuril to the drinking water of broiler chickens improved body weight gains. Bedrnik (1983) has established that a reduced efficacy of an anticoccidial drug always has an adverse effect on weight gain and/or on the feed conversion. These factors play a decisive part in practice, since they determine the economic success of the operation (Stephan et al., 1997).

Group D had the highest mean fecal score of 3.4 while groups A, B and C had much lower scores of 1.6, 1.8 and 1.4 respectively (Table I). Toltrazuril was thus most effective in reducing the deviation of the appearance of the droppings from normal, followed by amprolium and Embazine Fort in that order. This is in agreement with previous studies on the anticoccidial activity of toltrazuril (Stephan et al., 1997; Ramadan et al., 1997).

At day 21 of age (day 7 P.I.), the mean PCV of groups A (25.64), B (26.90), C (26.38), and D (23.59) birds were significantly lower ($P<0.05$) than that of the uninfected/unmedicated group E (28.06) birds (Table II). This shows the experimental infection resulted in anemia. Anemia due to intestinal destruction and resulting blood loss is a consistent finding in *Eimeria* species infection in chickens (McDougald and Reid, 1997). Treatment with amprolium, Embazine Fort® and toltrazuril in groups A, B and C respectively resulted in improvements in mean PCV when compared to group D birds. The untreated infected group D birds had a significantly lower ($p<0.05$) PCV than the treated groups A, B and C birds. At day 21 of age (day 7 P.I.) there was no significant difference ($p<0.05$) in PCV between the groups A, B and C birds.

In summary, the mean weight gains and PCV of chickens challenged with mixed *Eimeria* species oocysts and medicated with amprolium, Embazine Fort® and toltrazuril were significantly higher ($P < 0.05$) than the unmedicated infected controls. The lower mortality rate and fecal scores of medicated birds also suggested anticoccidial efficacy. Although amprolium, and Embazine Fort® were effective, treatment with toltrazuril resulted in significantly higher bodyweight gains than either amprolium or Embazine Fort®. Based on this, toltrazuril thus had higher efficacy than amprolium or Embazine Fort®. This could be due to the fact that amprolium and sulphonamides (including Embazine Fort®) have been in use in Nigeria for over two decades and resistance to them has been claimed. On the converse toltrazuril is a more recently introduced drug to Nigeria and is therefore less likely to show resistance. Toltrazuril may also be more efficacious than amprolium and Embazine Forte® because it is only administered for 2 days, unlike the other two drugs that are administered for 5 days. There is thus a higher chance of under dosing amprolium or Embazine Fort® and subsequent development of resistance.

CONCLUSION

Although amprolium and Embazine Fort® were efficacious, toltrazuril was superior and is thus recommended for therapy of coccidiosis in broiler chickens in Nigeria.

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